PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 417/06 — A1

(11) International Publication Number: **WO 99/54330**

(43) International Publication Date: 28 October 1999 (28.10.99)

(21) International Application Number: PCT/US99/08114

(22) International Filing Date: 14 April 1999 (14.04.99)

(30) Priority Data:
60/082,562 21 April 1998 (21.04.98) US

(71) Applicant: BRISTOL–MYERS SQUIBB COMPANY [US/US]; P.O. Box 4000, Princeton, NJ 08543–4000 (US).

(72) Inventors: VITE, Gregory, D.; 28 Continental Lane, Titusville, NJ 08560 (US). BORZILLERI, Robert, M.; 15232 Marie Court, Lawrenceville, NJ 08648 (US). HOFLE, Gerhard; Alter Weg 12a, D–38124 Braunschweig (DE). LEIBOLD, Thomas; In den Lindendoehren 38, D–38300 Braunschweig (DE).

(74) Agents: HOFFMAN, Frank et al.; Bristol–Myers Squibb Company, P.O. Box 4000, Princeton, NJ 08543–4000 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: 2,3–OLEFINIC EPOTHILONE DERIVATIVES

(57) Abstract

The present invention relates to 2,3–olefinic epothilone derivatives, methods of preparation of the derivatives and intermediates thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

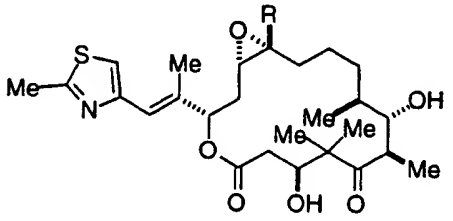
2,3-OLEFINIC EPOTHILONE DERIVATIVES

Field of the Invention

The present invention relates to epothilone derivatives, methods for the preparation of the derivatives and intermediates therefor.

Background of the Invention

Epothilones are macrolide compounds which find utility in the pharmaceutical field. For example, Epothilones A and B having the structures:



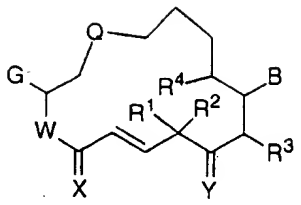
Epothilone A R = H

Epothilone B R = Me

have been found to exert microtubule-stabilizing effects similar to TAXOL and hence cytotoxic activity against rapidly proliferating cells, such as, tumor cells or other hyperproliferative cellular disease, see Angew. Chem. Int. Ed. Engl., 1996, 35, No. 13/14.

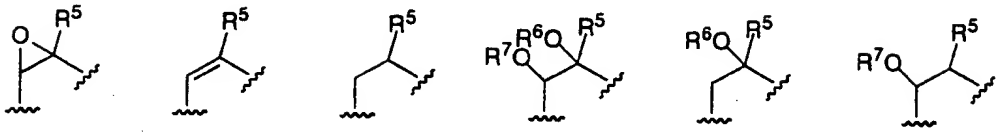
Summary of the Invention

The present invention relates to compounds of the formula

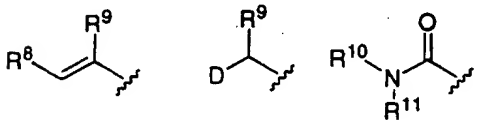


I

Q is selected from the group consisting of



G is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo,



;

W is O or N R_{12};

X is O, S, or H, H;

Y is selected from the group consisting of O; H, OR_{13}; OR_{14}, OR_{14}; NOR_{15}; H, NOR_{16}; H, $NR_{17}R_{18}$; H, H; or CHR_{19}; OR_{14} OR_{14} can be a cyclic ketal;

B is selected from the group consisting of H, OR_{20}, or $OCOR_{21}$, and $NR_{22}R_{23}$;

D is selected from the group consisting of $NR_{24}R_{25}$ or saturated heterocycle (such as piperidinyl, pyrrolidinyl, and the like);

R_1, R_2, R_3, and R_4 are selected from H or lower alkyl;

R_{15}, R_{16}, R_{17}, R_{18}, and R_{19} are selected from the group H, alkyl, substituted alkyl, or aryl;

R_6, R_7, R_{13}, R_{14}, R_{20}, and R_{21} are selected from the group H, alkyl, or substituted alkyl;

R_5, R_8, R_9, R_{22}, R_{24}, R_{26}, and R_{27} are selected from the group consisting of H, alkyl, substituted alkyl, aryl, heteroaryl, cycloalkyl, or heterocyclo;

R_{12}, R_{23}, and R_{25} are selected from the group consisting of H, alkyl, substituted alkyl, aryl, heteroaryl, cycloalkyl, heterocyclo, $R_{26}C{=}O$, $R_{27}SO_2$, hydroxy, O-alkyl or O-substituted alkyl;

and any salts, solvates or hydrates thereof.

Proviso

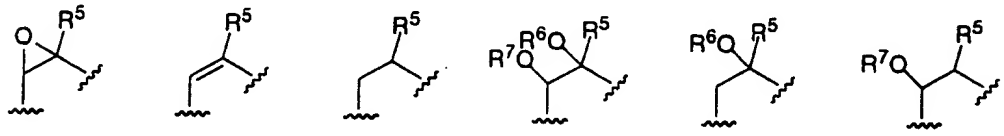
The present invention does not include compounds of formula I wherein

W and X are both O; and

R_1, R_2, R_3, R_4 are methyl; and

R_5 is H or methyl; and G is 1-methyl-2-(2-methyl-4-thiazolyl)ethenyl; and

Q is



Detailed Description of the Invention

Listed below are definitions of various terms used to describe this invention. These definitions apply to the terms as they are used

throughout this specification. unless otherwise limited in specific instances, either individually or as part of a larger group.

The term "alkyl" refers to straight or branched chain unsubstituted hydrocarbon groups of 1 to 20 carbon atoms, preferably 1 to 7 carbon atoms. The expression "lower alkyl" refers to unsubstituted alkyl groups of 1 to 4 carbon atoms.

The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyoxy, heterocylooxy, oxo, alkanoyl, aryloxy, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amines in which the 2 amino substituents are selected from alkyl, aryl or aralkyl, alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g. SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g. $CONH_2$), substituted carbamyl (e.g. CONH alkyl, CONH aryl, CONH aralkyl or cases where there are two substituents on the nitrogen selected from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and heterocyclos, such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Where noted above where the substituent is further substituted it will be with halogen, alkyl, alkoxy, aryl or aralkyl.

The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having 6 to 12 carbon atoms in the ring portion, such as phenyl, naphthyl, biphenyl and diphenyl groups, each of which may be substituted.

The term "aralkyl" refers to an aryl group bonded directly through an alkyl group, such as benzyl.

The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, phenyl, substituted phenyl, heterocyclo, halo, trifluoromethoxy, trifluoromethyl, hydroxy, alkoxy, cycloalkyloxy, heterocyclooxy, alkanoyl, alkanoyloxy, amino, alkylamino, aralkylamino, cycloalkylamino, heterocycloamino, dialkylamino, alkanoylamino, thiol, alkylthio, cycloalkylthio, heterocyclothio, ureido, nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthiono, arylthiono, alkysulfonyl, sulfonamido, aryloxy and the like. The substituent may be further substituted by halo. hydroxy, alkyl, alkoxy, aryl, substituted aryl, substituted alkyl or aralkyl.

The term "cycloalkyl" refers to optionally substituted, saturated cyclic hydrocarbon ring systems, preferably containing 1 to 3 rings and 3 to 7 carbons per ring which may be further fused with an unsaturated C_3-C_7 carbocyclic ring. Exemplary groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

The terms "heterocycle", "heterocyclic" and "heterocyclo" refer to an optionally substituted, fully saturated or unsaturated, aromatic or nonaromatic cyclic group, for example, which is a 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms

may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom.

Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1, 1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, and triazolyl, and the like.

Exemplary bicyclic heterocyclic groups include benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,1-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

Exemplary substituents include one or more alkyl groups as described above or one or more groups described above as alkyl substituents. Also included are smaller heterocyclos, such as, epoxides and aziridines.

The term "heteroatoms" shall include oxygen, sulfur and nitrogen.

The compounds of formula I may form salts with alkali metals such as sodium, potassium and lithium, with alkaline earth metals such as calcium and magnesium, with organic bases such as dicyclohexylamine, tributylamine, pyridine and amino acids such as arginine, lysine and the like. Such salts can be obtained, for example, by exchanging the carboxylic acid protons, if they contain a carboxylic acid, in compounds of formula I with the desired ion in a medium in which the salt precipitates or in an aqueous medium followed by evaporation. Other salts can be formed as known to those skilled in the art.

The compounds for formula I form salts with a variety of organic and inorganic acids. Such salts include those formed with hydrogen chloride, hydrogen bromide, methanesulfonic acid, hydroxyethanesulfonic acid, sulfuric acid, acetic acid, trifluoroacetic acid, maleic acid, benzenesulfonic acid, toluenesulfonic acid and various others (e.g., nitrates, phosphates, borates, tartrates, citrates, succinates, benzoates, ascorbates, salicylates and the like). Such salts are formed by reacting a compound of formula V in an equivalent amount of the acid in a medium in which the salt precipitates or in an aqueous medium followed by evaporation.

In addition, zwitterions ("inner salts") are formed.

Compounds of the formula I may also have prodrug forms. Any compound that will be converted in vivo to provide the bioactive agent (i.e., the compound for formula I) is a prodrug within the scope and spirit of the invention.

For example compounds of the formula I may form a carboxylate ester moiety. The carboxylate esters are conveniently formed by esterifying any of the carboxylic acid functionalities found on the disclosed ring structure(s).

Various forms of prodrugs are well known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol.42, p. 309-396, edited by K. Widder, et al. (Acamedic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krosgaard-Larsen and H. Bundgaard, Chapter 5, "Design and Application of Prodrugs," by H. Bundgaard, p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, et al., Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, et al., Chem Phar Bull, 32, 692 (1984).

It should further be understood that solvates (e.g., hydrates) of the compounds of formula I are also within the scope of the present invention. Methods of solvation are generally known in the art.

Use and Utility

The compounds of formula I are microtubule-stabilizing agents. They are thus useful in the treatment of a variety of cancers or other abnormal proliferative diseases, including (but not limited to) the following;

- carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma;
- hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma;
- hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia;
- tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma;
- other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma;
- tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas;
- tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and
- other tumors, including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma.

Compounds of formula I may also inhibit tumor angiogenesis, thereby affecting abnormal cellular proliferation. Such anti-angiogenesis properties of the compounds of formula I may also be useful in the treatment of certain forms of blindness related to retinal vascularization, arthritis, especially inflammatory arthritis, multiple sclerosis, restinosis and psoriasis.

Compounds of formula I may induce or inhibit apoptosis, a physiological cell death process critical for normal development and homeostasis. Alterations of apoptotic pathways contribute to the

pathogenesis of a variety of human diseases. Compounds of formula I, as modulators of apoptosis. will be useful in the treatment of a variety of human diseases with aberrations in apoptosis including cancer (particularly, but not limited to follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostrate and ovary, and precancerous lesions such as familial adenomatous polyposis), viral infections (including but not limited to herpesvirus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus), autoimmune diseases (including but not limited to systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel diseases and autoimmune diabetes mellitus), neurodegenerative disorders (including but not limited to Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration), AIDS, myelodysplastic syndromes, aplastic anemia, ischemic injury associated myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol induced liver diseases, hematological diseases (including but not limited to chronic anemia and aplastic anemia), degenerative diseases of the musculoskeletal system (including but not limited to osteoporosis and arthritis), aspirin-sensitive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases, and cancer pain.

The compounds of this invention are also useful in combination with known anti-cancer and cytotoxic agents and treatments, including radiation. If formulated as a fixed dose, such combination products employ the compounds of this invention within the dosage range described below and the other pharmaceutically active agent within its approved dosage range. Compounds of formula I can be used sequentially with known anticancer or cytotoxic agents and treatment, including radiation when a

combination formulation is inappropriate. Especially useful are cytotoxic drug combinations wherein the second drug chosen acts in a different phase of the cell cycle, e.g. S phase, than the present compounds of formula I which exert their effects at the G_2-M phase.

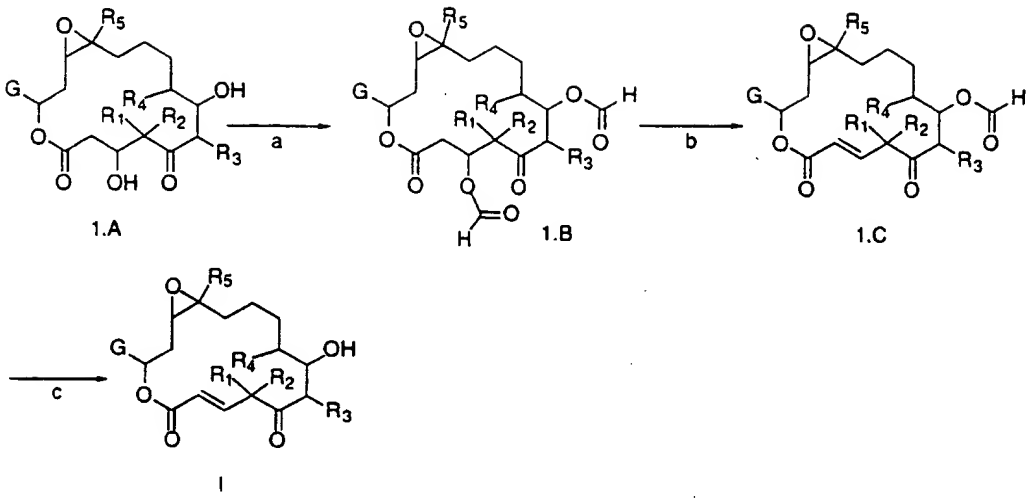
The present compounds may exist as multiple optical, geometric, and stereoisomers. Included within the present invention are all such isomers and mixtures thereof.

The compounds of this invention can be formulated with a pharmaceutical vehicle or diluent for oral, intravenous or subcutaneous administration. The pharmaceutical composition can be formulated in a classical manner using solid or liquid vehicles, diluents and additives appropriate to the desired mode of administration. Orally, the compounds can be administered in the form of tablets, capsules, granules, powders and the like. The compounds are administered in a dosage range of about 0.05 to 200 mg/kg/day, preferably less than 100 mg/kg/day, in a single dose or in 2 to 4 divided doses.

Method of Preparation

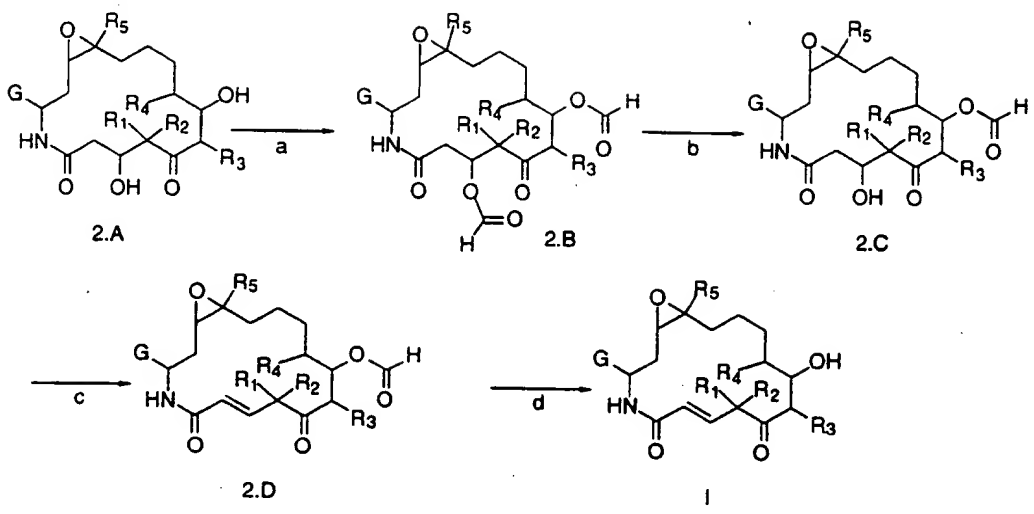
A compound of formula I can be prepared as shown in Scheme 1, using procedures described in PCT/EP96/05080. A compound of formula 1.A can be esterified using, for example, a mixture of formic acid and acetic anhydride to give a corresponding diformate 1.B. A compound of formula 1.C can be prepared from a compound of formula 1.B using a base such as DBU. A compound of formula I can be prepared from a compound of formula 1.C by treatment with methanolic ammonia.

Scheme 1



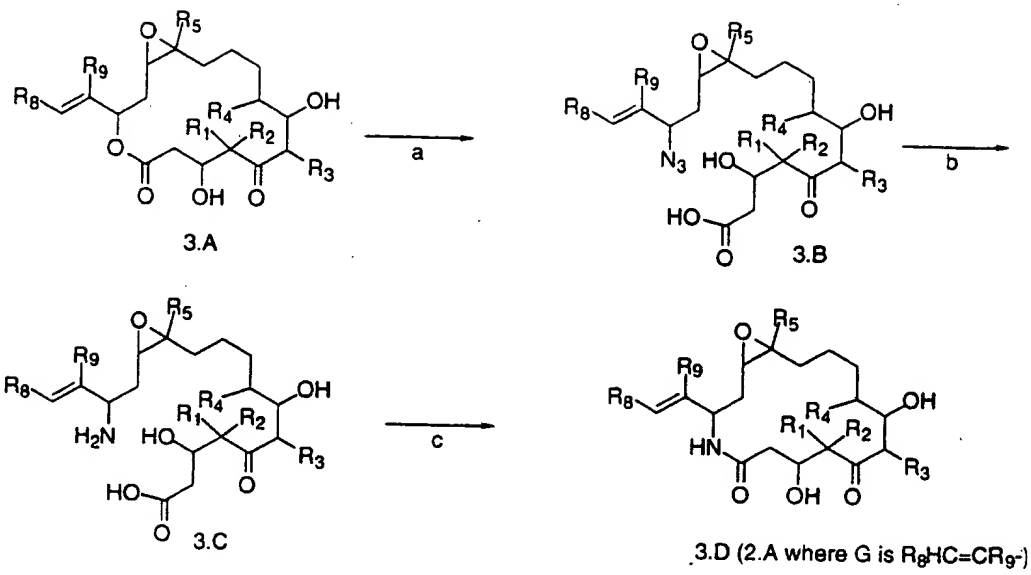
Compounds of formula I where X is O, W is NH, and Q is an oxiranyl group can be prepared as shown in Scheme 2. A compound of formula 2.A can be esterified using, for example, a mixture of formic acid and acetic anhydride to give a corresponding diformate 2.B. A compound of formula 2.C can be prepared from a compound of formula 2.B by treatment with a base such as DBU. A compound of formula 2.D can be prepared from a compound of formula 2.C using for example methanesulfonyl chloride and triethylamine. or Burgess' reagent. Treatment of a compound of formula 2.D with methanolic ammonia affords a compound of formula I where X is NH and Q is an oxiranyl group.

Scheme 2



A compound of formula 2.A where G is $-CR_9=CR_8H$ can be prepared as shown in Scheme 3. A compound of formula 3.B can be prepared from a compound of formula 3.A (an epothilone or epothilone-related natural product) by formation of pi-allylpalladium complex using, for example, palladium tetrakistriphenylphosphine followed by treatment with sodium azide (see, for example: Murahashi, S.-I.; et. al., J. Org. Chem. 1989, 54, 3292). Subsequent reduction of a compound of formula 3.B with a reducing agent such as triphenylphosphine provides a compound of formula 3.C. A compound of formula 3.D (or 2.A where G is $-CR_9=CR_8H$) can be prepared from a compound of formula 3.C by macrolactamization using, for example, diphenylphosphoryl azide (DPPA) or bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP).

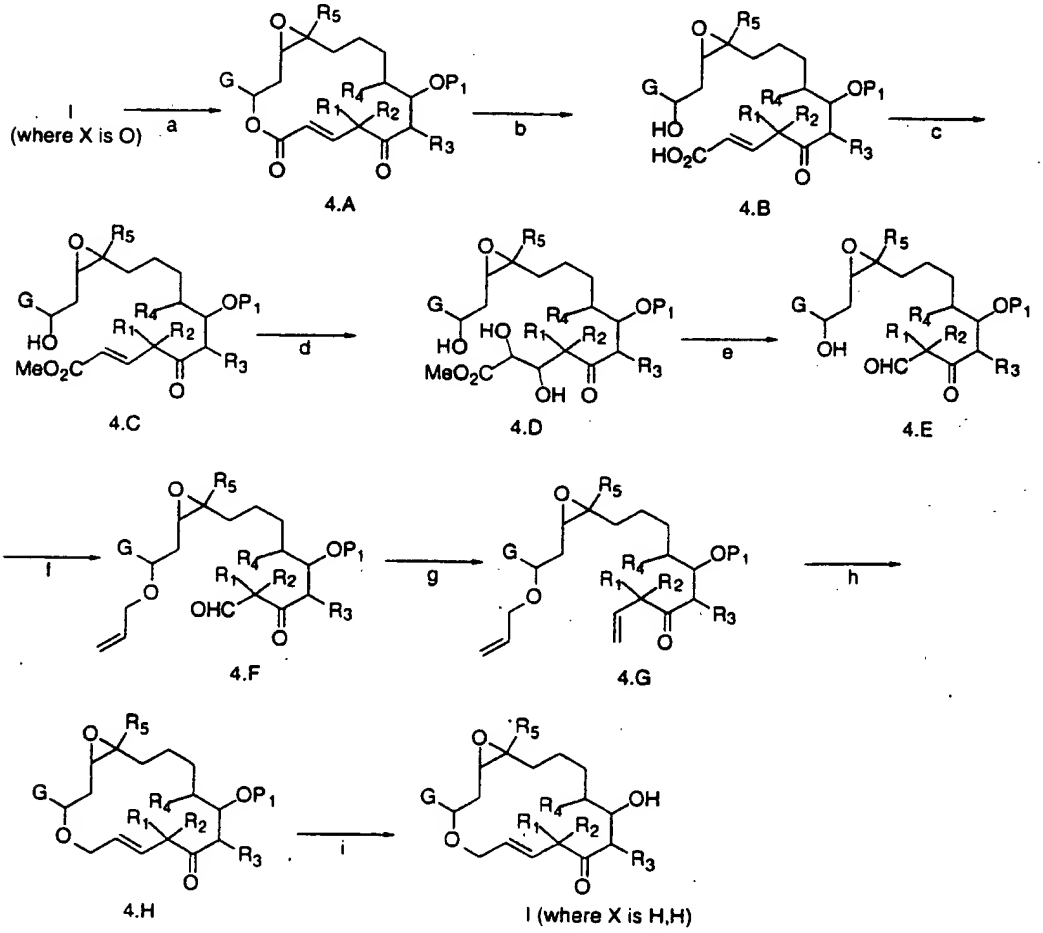
Scheme 3



A compound of formula I where W is O and X is H,H can be prepared as shown in Scheme 4. The alcohol moiety of a compound of formula I where both W and X are O can be protected using methods in the art to give a compound of formula 4.A, where P_1 is a suitable O-protecting group such as triethylsilyl. Hydrolysis of a compound of formula 4.A, using for example lithium hydroxide monohydrate, provides a compound of formula 4.B. Esterification of a compound of formula 4.B. using for example trimethylsilyl diazomethane, provides a compound of formula 4.C. Selective dihydroxylation of the α,β-unsaturated ester moiety of a compound of formula 4.C by known methods (see Sharpless, K.B. et al., J. Org. Chem. (1992) 57, 2768) provides a compound of formula 4.D. Oxidative cleavage of the diol of a compound of formula 4.D, using for example lead tetraacetate provides a compound of formula 4.E. A compound of formula 4.F can be prepared from a compound of formula 4.E using an allylating agent such as ally bromide and a silver salt such as silver oxide. A compound of formula 4.G can be prepared from a compound

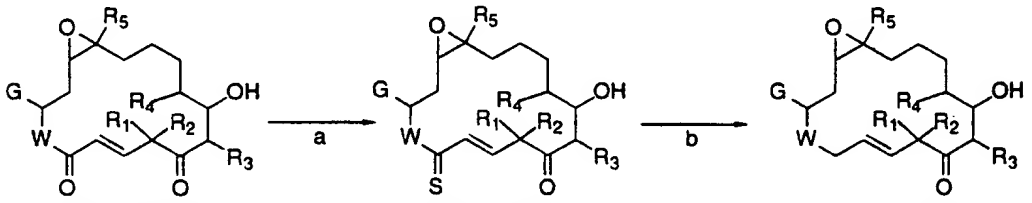
of formula 4.F using an olefinating agent such as methyltriphenylphosphonium bromide and a base such as sodium hexamethyldisilazide. A compound of formula 4.H can be prepared from a compound of formula 4.G by ring-closing metathesis using either the Grubbs ($RuCl_2$ (= CHPh)$(PCY_3)_2$; see Grubbs, R.H., et al., Angew. Chem. Int. Ed. Engl.; (1995) 34, 2039) or Schrock catalysts (see Schrock, R.R., et al., J. Am. Chem. Soc., (1990) 112, 3875). A compound of formula I where W is O and X is H,H can be prepared from a compound of formula 4.H by removal of the protecting group using for example acetic acid/THF/water mixtures.

Scheme 4



Alternatively, compounds of formula I where X is H,H can be prepared as shown is Scheme 5. A compound of formula I where X is S can be prepared from a compound of formula I where X is O using, for example, Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide]. A compound of formula I where X is H,H can be prepared from a compound of formula I where X is S by reduction with reducing agents such as tri-n-butyltin hydride, Raney nickel, or nickel boride. In Scheme 5, the hydroxyl group can be optionally protected using, for example, a triethylsily group which can be removed ultimately by treatment with hydrogen fluoride-pyridine or acetic acid/THF/water mixtures.

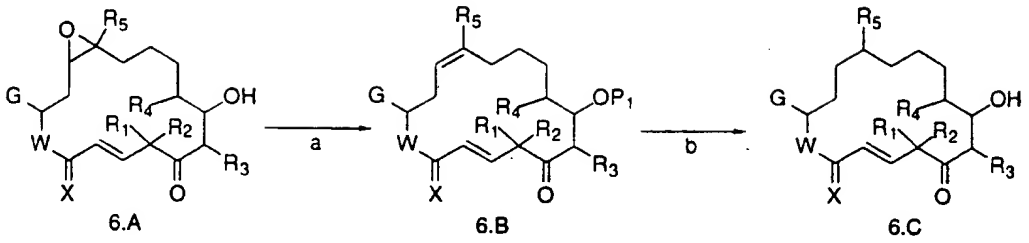
Scheme 5



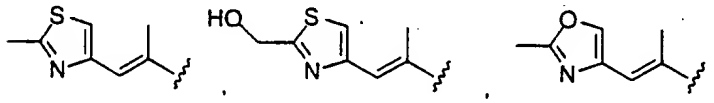
I (where X is O) I (where X is S) I (where X is H,H)

Compounds of formula I where Q is an olefinic group or the corresponding saturated derivative can be prepared as shown in Scheme 6. Compounds of formula I where Q is an oxiranyl group (i.e., compound 6.A) can be reduced using reagents such as reactive titanocene or tungsten chloride and butyllithium to provide compounds of formula I where Q is an olefinic group (i.e., compound 6.B). Further reduction using, for example, diimide provides compounds of formula I where Q is a saturated alkyl chain (i.e., compound 6.C).

Scheme 6

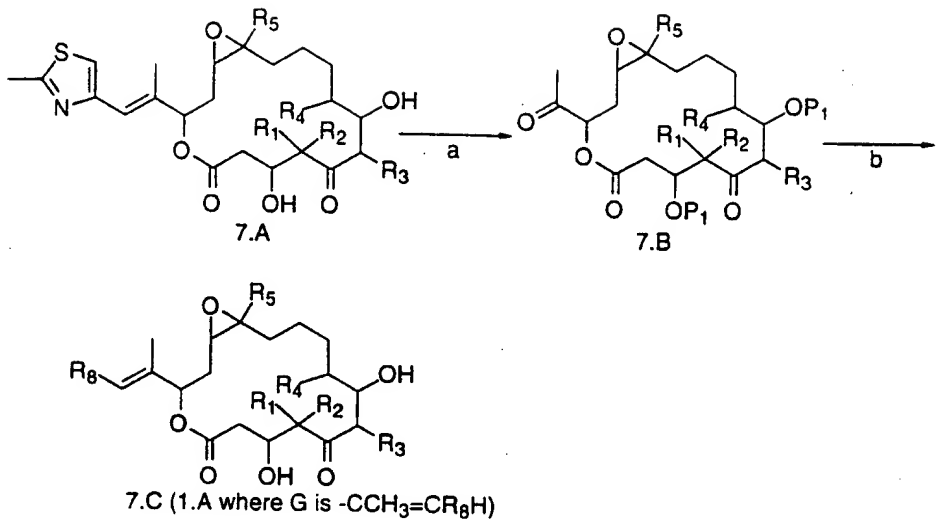


In Schemes 1, 3, 4, and 5, the starting material can be obtained from fermentation of *Sorangium cellulosum* as previously described (see Angew. Chem. Int. Ed. Engl., 1996, 35, No. 13/14.). In these fermentation products G is usually, but not exclusively, selected from the following:



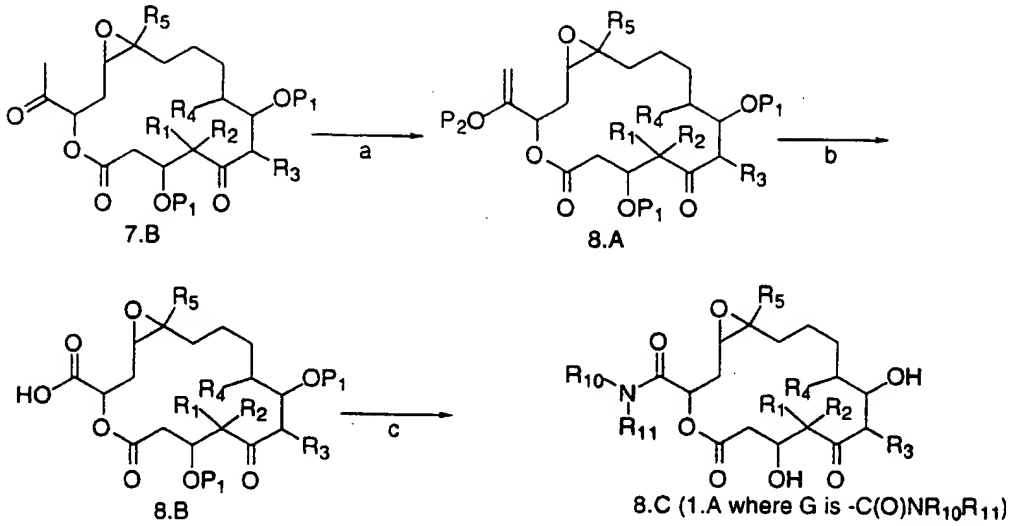
In cases where G is not selected from the preceding list or obtained from fermentation, synthetic methods can be used. For example, total synthesis routes have been described (See, for example: Danishefsky, S.J., et. al., J. Am. Chem. Soc., (1997) 119, 10073), and these methods can be used to provide compounds of formula 1.A where G is, for example, alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo, and $-CR_9=CR_8H$. In addition, semi-synthesis which utilizes degradation of natural epothilones can be employed. For example, epothilones (i.e., 7.A) can be protected and then degraded to a compound of formula 7.B (see PCT/EP96/05080). Subsequent olefination and deprotection provides compounds of formula 1.A where is $-CCH_3=CR_8H$ (i.e., compound 7.C). Alternatively, 7.B can be treated with an alkyl or arylmagnesium halide to provide a tertiary alcohol which can be dehydrated using, for example, Burgess reagent to provide a compound of formula 1.A where is $-CCH_3=CR_8H$ (i.e., compound 7.C).

Scheme 7



Furthermore, starting compounds of formula 1.A where G is -$C(O)NR_{10}R_{11}$ can be prepared from a compound of formula 7.B as shown in Scheme 8. A compound of formula 8.A where P is a trialkylsilyl group can be prepared from a compound of formula 7.B using for example t-butyldimethylsilyl chloride and triethylamine. Oxidative cleavage of a compound of formula 8.A using for example ozone provides a compound of formula 8.B. Amide coupling of a compound of formula 8.B using methods well known in the art followed by deprotection provides a compound of 1.A where G is -$C(O)NR_{10}R_{11}$ (i.e., compound 8.C).

Scheme 8



The *in vitro* assessment of biological activity of the compounds of formula I was performed as follows:

In vitro Tubulin Polymerization. Twice cycled (2X) calf brain tubulin was prepared following the procedure of Williams and Lee (see Williams, R.C., Jr., and Lee, J. C. Preparation of tubulin from brain. Methods in Enzymology 85, Pt. D: 376-385, 1982) and stored in liquid nitrogen before use. Quantification of tubulin polymerization potency is accomplished following a modified procedure of Swindell, et al., (see Swindell, C.S., Krauss, N.E., Horwitz, S.B., and Ringel, I. Biologically active taxol analogues with deleted A-ring side chain substituents and variable C-2' configurations. J. Med. Chem. 34: 1176-1184, 1991). These modifications, in part, result in the expression of tubulin polymerization potency as an effective concentration for any given compound. For this method, different concentrations of compound in polymerization buffer (0.1M MES, 1mM EGTA, 0.5 mM $MgCl_2$, pH 6.6) are added to tubulin in polymerization buffer at 37° in microcuvette wells of a Beckman (Beckman Instruments)

Model DU 7400 UV spectrophotometer. A final microtubule protein concentration of 1.0 mg/ml and compound concentration of generally 2.5, 5.0, and 10 μM are used. Initial slopes of OD change measured every 10 seconds were calculated by the program accompanying the instrument after initial and final times of the linear region encompussing at least 3 time points were manually defined. Under these conditions linear variances were generally $<10^{-6}$, slopes ranged from 0.03 to 0.002 absorbance unit/minute, and maximum absorbance was 0.15 absorbance units. Effective concentration ($EC_{0.01}$) is defined as the interpolated concentration capable of inducing an initial slope of 0.01 OD/minute rate and is calculated using the formula: $EC_{0.01}$ = concentration/slope. $EC_{0.01}$ values are expressed as the mean with standard deviation obtained from 3 different concentrations. $EC_{0.01}$ values for the compounds in this invention fall in the range 0.01-1000 μM.

Cytoxicity (In-Vitro)

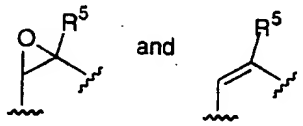
Cytoxicity was assessed in HCT-116 human colon carcinoma cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt) assay as reported in T.L. Riss, et. al., "Comparison of MTT, XTT, and a novel tetrazolium compound MTS for in vitro proliferation and chemosensitivity assays.," *Mol. Biol. Cell* 3 (Suppl.):184a, 1992. Cells were plated at 4,000 cell/well in 96 well microtiter plates and 24 hours later drugs were added and serial diluted. The cells were incubated at 37° form 72 hours at which time the tetrazolium dye, MTS at 333 μg/ml (final concentration), in combination with the electron coupling agent phenazine methosulfate at 25 μM (final concentration) was added. A dehydrogenase enzyme in live cells reduces the MTS to a form that absorbs light at 492nM which can be quantitated spectrophotometrically. The greater the absorbance the greater the

number of live cells. The results are expressed as an IC_{50}, which is the drug concentration required to inhibit cell proliferation (i.e. absorbance at 450nM) to 50% of that of untreated control cells. The IC_{50} values for compounds of this invention fall in the range 0.01 - 1000 nM.

Preferred Compounds

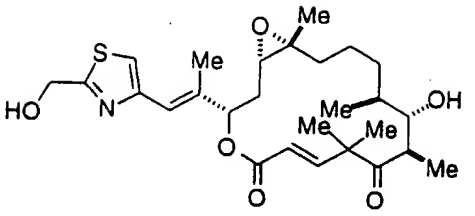
As preferred compounds of the present invention are compounds of formula I wherein

Q is selected from the group consisting of

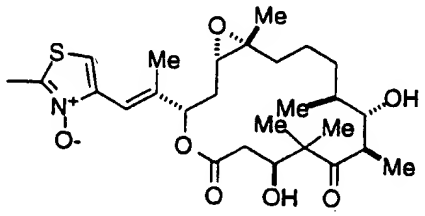


and y is oxygen.

Example 1



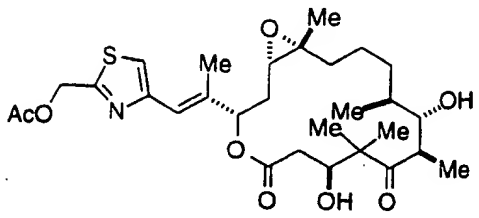
[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-hydroxymethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6(E)-ene-5,9-dione.



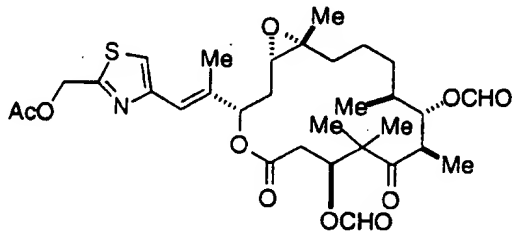
A. [1S-[1R*,3R*(E),7R*,10R*,11S*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione, *N*-oxide.

A solution of epothilone B (2.0 g, 3.9 mmol) in CH_2Cl_2 (30 mL) was treated with 3-chloroperoxybenzoic acid (1.0 g, 5.9 mmol) at 25 °C, under Ar for 2 h. An additional 0.5 g (3.0 mmol) of 3-chloroperoxybenzoic acid was added and the reaction mixture was then stirred for 2 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ (75 mL), 5 % aqueous Na_2SO_3 (75 mL), H_2O (75 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash chromatography

(SiO_2, 4.5 x 30 cm. 2-10 % MeOH-$CHCl_3$ gradient elution) to afford Compound A (1.04 g, 50 %) as a white solid. MS (ESI^+): 524.3 $(M+H)^+$; MS (ESI^-): 522.5 $(M-H)^-$.

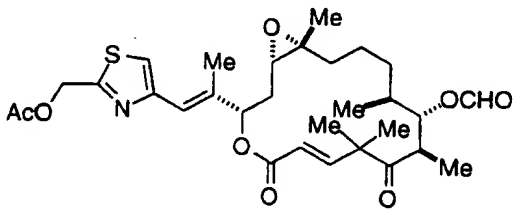


B. [1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-acetoxymethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione. To a resealable Kontes vial was added compound A (0.20 g, 0.38 mmol) and acetic anhydride (2 mL) under Ar. The reaction vessel was sealed under Ar and heated to 75 °C for 4 min. Acetic acid (0.4 mL) was then introduced into the reaction vessel and the reaction mixture was heated for an additional 30 min at 75 °C. After the Kontes vial was cooled to 25 °C, the volatiles were removed *in vacuo* and the residue was purified by flash chromatography (SiO_2, 3.0 x 15 cm, 45:45:10 hexane/*tert*-butyl methyl ether/MeOH) to afford Compound B (0.15 g, 68 %) as a colorless oil. MS (ESI^+): 566.2 $(M+H)^+$, 1131.5 $(2M+H)^+$; MS (ESI^-): 564.4 $(M-H)^-$, 1129.7 $(2M-H)^-$.



C. [1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-acetoxymethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione, 7,11 diformate.

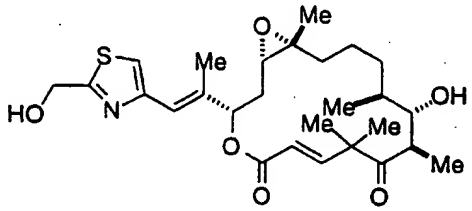
A solution of compound B (0.15 g, 0.27 mmol) in CH_2Cl_2 (5 mL) was treated with 4-*N,N*-dimethylaminopyridine (71 mg, 58 mmol), triethylamine (0.37 mL, 2.6 mmol), and formic acid (50 mL, 1.3 mmol) at 25 °C, under Ar. The reaction mixture was cooled to -15 °C and acetic anhydride (0.12 mL, 1.3 mmol) was added over 3 min. The reaction mixture was stirred at -15 °C (15 min), warmed to 25 °C (15 min), quenched with pH 7.0 phosphate buffer and extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with aqueous 1 N HCl (50 mL), 10 % aqueous $NaHCO_3$ solution (50 mL), brine (50 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, 1.5 x 10 cm, 10 % acetone-CH_2Cl_2) to afford Compound C (0.134 g, 84 %) as a glass. MS (ESI^+): 622.2 $(M+H)^+$.



D. [1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-acetoxymethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6(E)-ene-5,9-dione, 11-formate.

A solution of compound C (0.13 g, 0.21 mmol) in CH_2Cl_2 (2.2 mL) was treated with 1.8-diazabicyclo[5.4.0]undec-7-ene (0.31 mL, 2.1 mmol) at 25 °C, under Ar. The reaction mixture was stirred at 25 °C, for 2 h, quenched

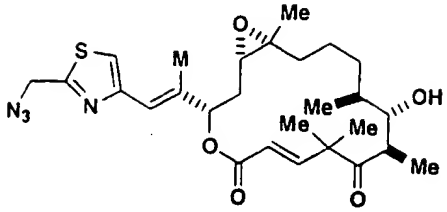
by the addition of pH 4.0 phosphate buffer, and extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (30 mL), brine (30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, 1.5 x 10 cm, 25-50 % EtOAc-hexane gradient elution) to afford Compound D (0.11 g, 92 %) as a foam. MS (ESI^+): 576.2 $(M+H)^+$.



E. [1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-hydroxymethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6(E)-ene-5,9-dione.

A solution of compound D (0.11 g, 0.19 mmol) in MeOH (1.0 mL) was treated with 2 M ammonia in methanol (1.0 mL) at 25 °C, under Ar. The reaction mixture was warmed to 45 °C for 1 h and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, 1.5 x 10 cm, 2-5 % MeOH-$CHCl_3$ gradient elution) to afford the title compound (95 mg, 98 %) as a white foam. MS (ESI^+): 506.2 $(M+H)^+$, 1011.3 $(2M+H)^+$; MS (ESI^-): 504.5 $(M-H)^-$.

Example 2



[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-azidomethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6(E)-ene-5,9-dione.

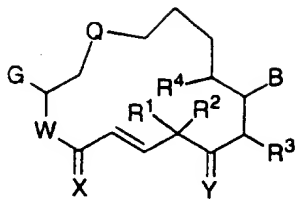
To a stirred solution of Compound 1E (3.0 mg, 0.0059 mmol) in 0.5 mL THF at
0C was added a 0.2M solution of diphenylphosphoryl azide (DPPA) in THF (35 µl, 0.0071 mmol, 1.2 eq) followed by addition of a 0.2M solution of DBU in THF (30 µL, 0.0060 mmol, 1eq). The mixture was allowed to stir at 0C for 3.5 h. An additional 15 µL of DPPA solution (0.0030 mmol, 0.5 eq) and 30 µL of DBU solution (0.0060 mmole, 1 eq) were added, and the mixture was allowed to stir at 0C for an additional 20
min. The solution was then warmed to 25C and allowed to stir for 15 h. The
mixture was diluted with 60 mL ethyl acetate then washed with 10 mL water and
dried over Na2SO4. The organic layer was concentrated in vacuo and purified by
silica gel chromatography using 2.5% MeOH in CHCl3 to afford 2 mg of a clear
film (65%). M+H = 531.2

Also produced following the procedure of Example 2 is the compound:

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-aminomethyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6(E)-ene-5,9-dione.

What is claimed.

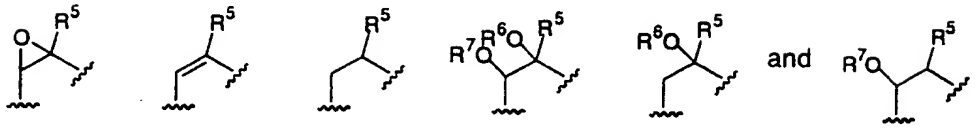
1. A compound of the formula



I

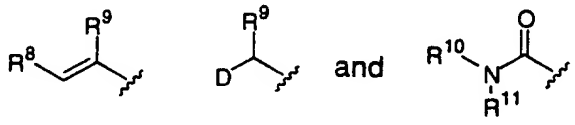
wherein

Q is selected from the group consisting of



G is selected from

G is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo,



W is O or N R_{12};

X is O, S, or H, H;

Y is selected from the group consisting of O; H, OR_{13}; OR_{14}, OR_{14}; NOR_{15}; H, NOR_{16}; H, $NR_{17}R_{18}$; H, H; or CHR_{19}; OR_{14} OR_{14} can be a cyclic ketal;

B is selected from the group consisting of H, OR_{20}, or $OCOR_{21}$, and $NR_{22}R_{23}$;

D is selected from the group consisting of $NR_{24}R_{25}$ and saturated heterocycle (such as piperidinyl, pyrrolidinyl, and the like);

R_1, R_2, R_3, and R_4 are selected from H or lower alkyl;

R_{15}, R_{16}, R_{17}, R_{18}, and R_{19} are selected from the group H, alkyl, substituted alkyl,and aryl;

R_6, R_7, R_{13}, R_{14}, R_{20}, and R_{21} are selected from the group H, alkyl, and substituted alkyl;

R_5, R_8, R_9, R_{22}, R_{24}, R_{26}, and R_{27} are selected from the group consisting of H, alkyl, substituted alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclo;

R_{12}, R_{23}, and R_{25} are selected from the group consisting of H, alkyl, substituted alkyl, aryl, heteroaryl, cycloalkyl, heterocyclo, $R_{26}C{=}O$, $R_{27}SO_2$, hydroxy, O-alkyl or O-substituted alkyl;

and any salts, solvates or hydrates thereof

with the proviso that together

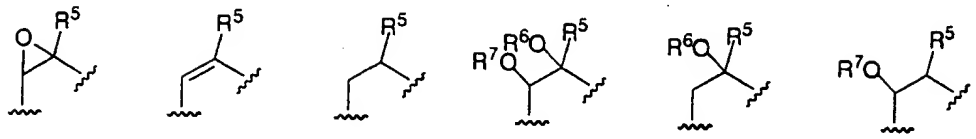
W and X as O;

R_1, R_2, R_3, R_4 as methyl;

R_5 as H or methyl;

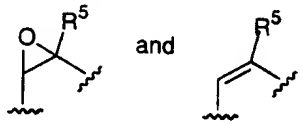
G as 1-methyl-2-(2-methyl-4-thiazolyl)ethenyl; and

Q as



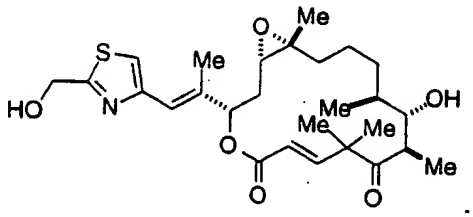
are excluded.

2. The compound of claim 1 wherein Q is selected from the group consisting of

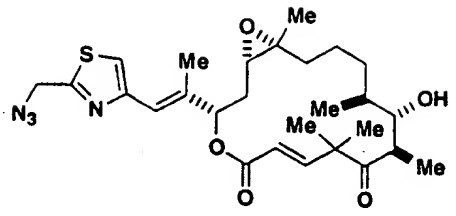


and y is oxygen.

3. A compound of the formula



4. A compound of the formula



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08114

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07D 417/06
US CL : 548/204

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/204

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICOLAOU, K.C. et al. An Approach to Epothilones Based on Olefin Metathesis. Angew. Chem. Int. Ed. Eng. 1996, Vol. 35, No. 20, pp. 2399-2401, see cpds 15-18.	1,2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 JUNE 1999	Date of mailing of the international search report 20 JUL 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT GERSTL Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★